Correlation between Photosynthesis and Plant Lipid Composition

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Thylakoid, Lipid Composition, P/2e Ratio

An attempt is reported to link changes in thylakoid lipid composition with the electron-transport and ATP-formation capability of the chloroplast. The observation that the monogalactolipid : phosphatid-ratio (MGDG/PL) of leaf sections shows a linear correlation with the P/2e value of simultaneously isolated Jensen-Bassham plastids was followed up experimentally for an *in vitro* plastid system. Lipid composition in these experiments was changed by illuminating the suspension with white light. The result shows, that similar dependencies can be obtained with both Jensen-Bassham chloroplasts and, up to a certain degree, chloroplast membrane fragments.

Introduction

Lipids of chloroplast membranes, necessary components of their structure and coherence, are not just static elements but possess a considerable dynamic of their own. The lipids are highly mobile within the membrane and both the overall content and the individual chemical species are quite variable within short periods of time. Heise and Stottmeister [1] suggested, that the observed diurnal oscillations in the lipid pattern of leaves seems to go parallel to the structural alterations of thylakoids in the time course of a day which were reported by Bartels [2]. The recent finding of Mazliak [3] that a considerable exchange of lipids takes place between the various cell organelles supports and at the same time complicates further the above notion.

During a study of daily variations in leaf lipid content in relation to the function of isolated chloroplasts we noticed an apparent linear correlation between the MGDG/PL ratio of the leaf sections and the P/2e value obtainable with the respective plastid preparation. This observation, depicted in figure 1, served as a starting point to explore further the functional interaction between the components of the electron transport system and the surrounding membrane composition. The results of this investigation are the topic of this paper.

Material and Methods

Both leaf sections and chloroplasts of spinach were used in the experiments. Whole chloroplasts of the Jensen-Bassham type were prepared as follows: isolation of plastids in 0.33 M sorbitol – 2 mM $NaNO_3$ – 2 mM EDTA – 2 mM Na-isoascorbate – 1 mM $MnCl_2$ – 1 mM $MgCl_2$ – 0.5 mM K_2HPO_4 – 20 mM NaCl – 50 mM MES pH 6.3; this was followed by one wash in a medium containing the same components except isoascorbate and resuspension to 1 mg/ml. The resuspension medium used 50 mM HEPES pH 6.7 instead of MES.

Class II chloroplasts, mostly thylakoid membrane fragments, were prepared according to Hesse *et al.* [4]. The isolation and first wash of the plastids occurred in 0.3 M sucrose – 50 mM NaCl – 1 mM $MgCl_2$ – 10 mM TRICINE pH 7.8; it was followed by 3 washes in 50 mM NaCl – 1 mM $MgCl_2$ – 2 mM TRICINE pH 8 and resuspension to 1 mg/ml in isolation medium.

The lipid composition of the chloroplast suspensions was altered in a straightforward way by illuminating dense plastid suspensions (1 mg/ml) for various amounts of time with white light (2×10^5 ergs/cm^2 s). No electron acceptor was present during illumination, which was carried out in a water bath at 20 °C under mechanical shaking to prevent settling of the suspension. Care was taken, that illumination was started simultaneously for all samples in the experimental set, removing at appropriate times samples for further analysis. Functional measurements were made immediately following this ligth exposure. Aliquots were drawn from the preilluminated samples, mixed with the respective assay media and chloroplast function was determined using set periods of 3 minutes illumination with actinic ligth.

Abbreviations: MGDG, monogalactosyl diglyceride; PL, total phospholipids; NL, lipid fraction containing acyl-glycerides, fatty acids and acylated steryl glycosides; DGDG, digalactosyl diglyceride.

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Electron transport was determined spectrophotometrically through the reduction of ferricyanide, concomitant ATP formation through a sligthly modified hexokinase test [5]. The hypotonic nature of the assay medium sufficed to achieve satisfactory rates also in the case of Jensen-Bassham type chloroplasts.

The main amount of the preilluminated samples was used for lipid determination. Leaf sections were killed in liquid nitrogen, ground and extracted according to Bligh and Dyer [6], chloroplast suspensions extracted directly using the same procedure. The extracted lipids were separated by thin – layer chromatography [7] and analyzed further as described before [1, 8]. The values of lipid concentration obtained differed within 30% due to the method used (acylester, glycerol, galactose or sugar based measurements). Standard variations within a particular method and a given preparation were between 6 – 8%.

Results

The data of Table I verify and extend the observation of figure 1. Essentially the same correlation is noticed in the chloroplasts isolated from preilluminated leaves. This strengthens the suggestion that the correlation between P/2e and MGDG/PL is not a superficial one. The higher values of MGDG/PL correlating to the same P/2e ratio in the chloroplasts have to be expected, since their membranes contain very little phosphatides.

Table I. Comparison of MGDG/PL-ratio and P/2e value; A) of whole leaves using the P/2e-quotient of simultaneously isolated chloroplasts, B) of isolated chloroplasts.

	A)			B)		
MGDG/PL	0.76	0.91	1.17	1.45	1.82	2.09
P/2e	0.59	0.76	1.04	0.84	0.94	1.23

The leaves for determination of lipids or plastid preparation were taken from a "daylight" growth chamber at random intervals.

The variations in lipid composition shown in Fig. 1 and Table I were induced in the *in vivo* system, *i. e.* by exposure of whole plants to various amounts of "daylight" in a controlled growth chamber. Fig. 2 A, however shows that the same effect can be achieved by illuminating fairly concentrated suspensions of Jensen-Bassham type plastids for various length of time. The immediately following assay for Hill activity and ATP-formation in saturating actinic light using an aliquot of this preilluminated sample leads again to the linear correlation between P/2e value and lipid ratio.

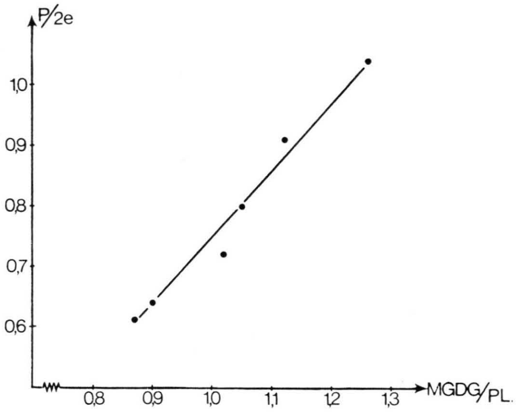

Fig. 1. Correlation of the MGDG/PL ratio, found in leaf sections of spinach, and the P/2e ratio of chloroplasts isolated from the same material. Every point originates from a different experiment.

Table II shows a detailed picture of the effect of illumination on these plastid suspensions. The MGDG concentration per chlorophyll increases within the first two minutes of light exposure while the phosphatides stay more or less constant. The linolenic acid content of the lipids more or less parallels their concentration (data not shown).

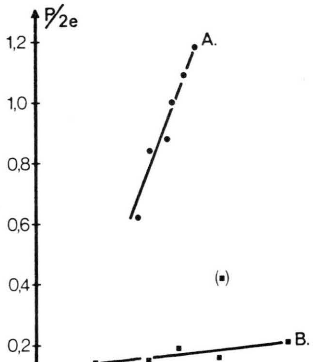

Fig. 2 A. Correlation between the MGDG/PL ratio, varied by illumination of a dense (1 mg/ml) plastid preparation of Jensen-Bassham type, and the P/2e ratio determined in a subsequent measurement. The data correspond to those of Table II.

Fig. 2 B. Correlation between the MGDG/PL ratio, varied by illumination of a dense (1 mg/ml) chloroplast membrane preparation, and the P/2e ratio determined in a subsequent measurement. The data correspond to those of Table III.

Table II. Correlation between light induced lipid changes in Jensen-Bassham type chloroplasts and their photosynthetic activity.

Time of illumination [min]	Lipid composition [μmol lipid/μmol Chl]					Photosynthetic activities		
	MGDG	DGDG	PL	NL	MGDG/PL	(1)	(2)	(3)
0	1.59	0.68	1.08	0.25	1.47	136	60	0.88
2	1.74	0.63	1.05	0.17	1.66	131	77	1.18
4	1.60	0.74	1.01	0.23	1.58	132	72	1.09
6	1.51	0.77	1.01	0.30	1.50	136	68	1.00
8	1.43	0.62	1.06	0.48	1.35	157	66	0.84
10	1.22	0.58	0.96	0.83	1.27	200	62	0.62

(1) e^--transport ($+ADP+P_i$) [μmol FeCy red/mg Chl×h];
(2) photophosphorylation [μmol ATP/mg Chl×h];
(3) P/2e-quotient.
See Methods for particulars. All values are averages of at least 5 independent experiments.

Table III. Correlation between light induced lipid changes in a chloroplast membrane preparation and their photosynthetic activity.

Time of illumination [min]	Lipid composition [μmol lipid/μmol Chl]					Photosynthetic activities		
	MGDG	DGDG	PL	NL	MGDG/PL	(1)	(2)	(3)
0	1.50	0.65	0.82	0.98	1.83	209	44	0.42
2	1.52	0.59	0.67	1.17	2.27	240	25	0.21
4	1.29	0.58	0.84	1.23	1.54	248	24	0.19
6	1.25	0.55	0.69	1.45	1.81	264	21	0.16
8	1.15	0.54	0.86	1.39	1.34	258	20	0.15
10	0.86	0.40	0.88	1.80	0.98	273	19	0.14

(1), (2) and (3) see Table II.
Particulars of the experiments given in Methods.
All values are averages of at least 4 independent experiments.

If there is indeed a correlation between MGDG/PL ratio and the P/2e value, as the aforegoing data indicate, these features should derive from the particular functional membrane, the thylakoid itself. Thus, one should observe this relationship also in thylakoid membranes possessing only the bare minimum of structure and function, *i. e.* fragments with only electron transport and photophosphorylation. In our experiments we used the chloroplasts prepared according to Hesse *et al.* [4].

Fig. 2 B, shows that also in this case the linear relation between MGDG/PL and P/2e is still noticeable. We interpret the rather flat slope of this curve, however, as indicating that this relation might not be as rigid as expected. Obviously, structural factors such as holes in the membrane etc. influence the results.

Table III gives the detailed alterations induced by illumination of the thylakoid membrane preparation. In contrast to the data obtained with Jensen-Bassham chloroplasts (Table II) the changes in lipid composition correlate relatively linear with time of light exposure in this case. The alteration of membrane composition through light is documented in both types of plastid preparation by the inverse behaviour of MGDG (decreasing) and NL (increasing). The linearity of these changes in thylakoid membrane preparations is probably entirely due to photochemical breakdown of lipid constituents *e. g.* $C_{18:3}$, a feature becoming obvious in the case of Jensen-Bassham plastids only after several minutes [9].

Discussion

It is safe to assume that the increase of electron transport and the simultaneous decrease in ATP formation during prolonged preillumination are due to uncoupling, which is thus an immediate consequence of the light induced alteration of the membrane MGDG/PL ratio. The inverse behaviour of NL compared to MGDG we interpret as showing that NL is either the product of MGDG breakdown or that the synthesis of MGDG from diglycerides is inhibited. The latter aspect is corroborated by experiments showing declining incorporation into the glycolipid – and increasing ^{14}C-incorporation into the NL-fraction of Jensen-Bassham chloroplasts [10].

There remains the question why the MGDG/PL ratio seems such a good indicator of the P/2e value, especially since the phosphatides are supposed to be predominantly non-chloroplast lipids originating from a contamination of the preparation by other organelles [11, 12]. This might be due to a small but significant portion of PL which is thylakoid specific. This notion is supported by the fact that trans-Δ_3-hexadecenoic acid, only found in this particular lipid, is used as specific marker for thylakoids [3].

In conclusion we suggest, that illumination of leaf sections or chloroplast suspensions induces a change in membrane properties which are reflected in, or even control, the P/2e ratio. We used the relation of MGDG/PL as a reliable indicator only, due to the reasons stated above. Similar predictions can be made, however, with the MGDG/NL ratio, as a check of Table II and Table III will show. We did not emphasize this point, since the analytical technique used does not allow to differentiate the NL-fraction into the different acyl glycerides proper and the contaminating acylated steryl glycosides and free fatty acids. Thus we did not establish the possible error margin in NL and a definite involvement of these compounds in the effects described. We want to point out in this context that not only long term storage of leaf homogenates leads to lipid artefacts, but that this holds true also for isolated chloroplasts [13, 14]. Finally we want to mention the observation that the MGDG/PL *vs.* P/2e relation seems to hold also for an in vivo system, as shown in Fig. 1, which constitutes in our opinion added support for an active control between the functional properties of thylakoids and their membrane lipid composition.

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[1] K.-P. Heise and A. Stottmeister, Ber. Dtsch. Bot. Ges. **89,** 677–694 (1976).
[2] F. Barthels, Protoplasma **72,** 27–41 (1971).
[3] P. Mazliak, Lipids and Lipid Polymers in Higher Plants, pp. 48–74 (M. Tevini and H. K. Lichtenthaler, eds.), Springer, Berlin 1977.
[4] H. Hesse, F. Jank-Ladwig, and H. Strotmann, Z. Naturforsch. **31 c,** 445–451 (1976).
[5] D. Geller, Methods in Enzymology **XXIV,** pp. 88–92 (A. San Pietro, ed.), Academic Press, New York 1972.
[6] E. G. Bligh and W. J. Dyer, Can. J. Biochem. **37,** 911–917 (1959).
[7] P. Pohl, H. Glasl, and H. Wagner, J. Chromatogr. **49,** 488–492 (1970).
[8] K.-P. Heise and G. Jacobi, Z. Naturforsch. **28,** 120–127 (1973).
[9] G. Harnischfeger, Planta **104,** 316–328 (1972).
[10] K.-P. Heise, Abstract Symposium: "Recent Advances in the Biochemistry and Physiology of Plant Lipids", Göteborg 1978.
[11] L. R. O. Mackender and R. M. Leech, Plant Physiol. **53,** 496–502 (1974).
[12] K.-P. Heise and G. Krapf, Z. Naturforsch. **32 c,** 611–616 (1977).
[13] J. F. G. M. Wintermans, Biochemistry of Chloroplasts **Vol. 1,** p. 116 (T. W. Goodwin, ed.), Academic Press, London and New York 1966.
[14] S. Hoshina and K. Nishida, Plant Cell Physiol. **16,** 465–474 and 475–484 (1975).